Department MOL. BIOL - PROT. EXPR.
Subject 12/95 - 6/5/95
Name ANN KIM #9
Address

43-648
National Brand
Computation Notebook
Dennison Stationery Products Co., Framingham, MA 01701
75 Sheets
11¼" x 9¼"
4x4 Quad.
0 73333 43648 8

Ruben EXHIBIT #92

Department Mol. Biol - Prot. Expr.

Subject 12/95 - 6/5/95

Name Ann Kim #9

Address

National Brand 43-648

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701

75 Sheets
11¾" x 9¼"
4x4 Quad.

0 73333 43648 8

2

Pg 151 Book 8 #236

1/24/95

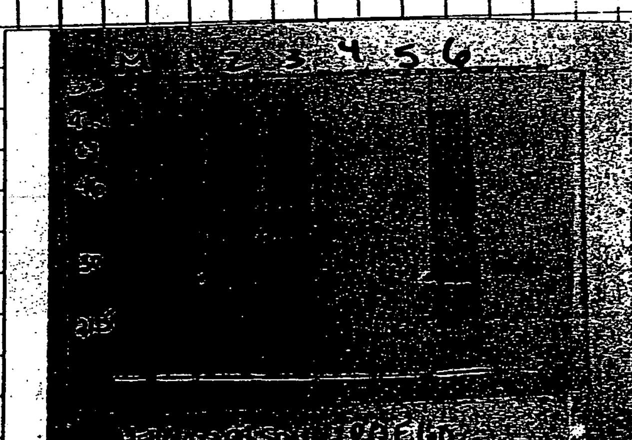
Spin HTPAN08 185 bp + PQE60 in
6M Gn HCl pH 8 - 8K 20 min.

Equilibrate Ni SO4 column with pH8
6M Gn HCl

Apply Supernatant to Column - Collect Flow
Wash 30 ml pH8 6M Gn HCl - Collect pH8
Wash 30 ml pH6 6M Gn HCl - Collect pH6
Elute 5 ml pH5 6M Gn HCl - Collect pH5
Strip 30 ml pH2 6M Gn HCl - Collect pH2.

Add 50 µl of collected material
to 450 µl H2O
50 µl 0.15% NaDOC
75 µl 50% TCA.

Mix well
Spin 5 min
Remove supernatant
Resuspend pellet in 15 µl 0.2N NaOH / 15 µl 2X Buff
Heat 100°C. 5 min
Run 20 µl on gel with Rainbow Marker.
on 12.5% gel at 150V 1 1/2 hrs.
Stain 1/2 hr
De Stain 1 1/2 hr.



1 Uninduced
2 1 Crude Extract
3 2 flow
4 3 pH8
5 4 pH6
6 5 pH5
7 6 pH2

185 bp

Dialyze & Send some to protein exp to mature our column.

Hank R. 1/26/95

11-6 PQE60 HTPAN0618 85bp+PQE60

(pg 148 Book 8 #236)

2/2/95

11-6 - Reapply to column & Strip again to try & Purify more

Start Dialyzing ~~[illegible]~~ 2ml in Dialysis Tubing

3M Gn HCl / Hepes 5hrs.
1.5M Gn HCl / Hepes Overnight

Reapply 43ml to Ni SO4 Column - to Renature over column - of HTPAN08 185bp+PQE60.

2/3/95

Change Buffer:

1M Gn HCl / Hepes 6hrs.
0.5M Gn HCl / Hepes Over Weekend

Carrie will finish

2/6 - 2/10 Vacation!

2/13/95

HTPAN08 185bp + PQE60

Strip Column that has ~~[illegible]~~ renatured - Protein in 2 Strips in Immidazole Elution Buffer: 50mM NaPO4 pH 6
250 mM Imidizole
300 mM NaCl
10% Glycerol

2 Strips at 2.5ml each.

Run on Stacking gel with LMW Marker - 12.5% gel 150 V.

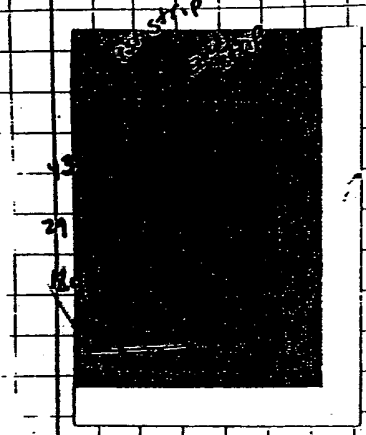
Stain 1/2 hr
destain 1 hr.

4 IL-6 PQE60 || HTPA1OB 185bp + PQE60

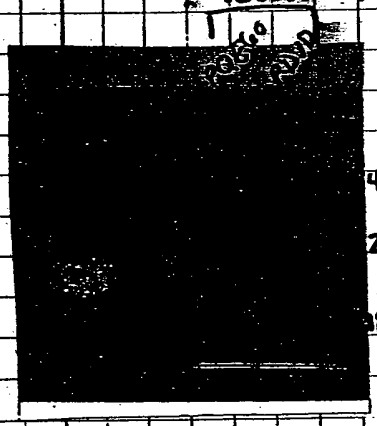
2/3/95

HTPA1OB 185bp + PQE60

Store 4°C.



IL-6 PQE60+pDD 2nd Coleumn Extraction



HTPAN08 / HTPB411 - PD10 - PA2 [redacted]

pg 152 Book 8 # 236

1/26/95

Received Primers for HTPAN08 & HTPB411
Repatect 55°C O/N.

1/27/95

PCR Fragments.

HTPB411 PA2.

9130 5' Bam.	1
3' Xba New.	1
10x dNTP	50
10x PCR.	50
Taq	2
H_2O	397
DNA (10ng/ul)	1
	500ul - 100ul / reaction.

HTPAN08.

	51 PA2	185 PA2	51 PD10	185 PD10
3146	2	2	2	2
9111:	20	9112: 20	9113: 24	9114: 20
10x dNTP	50	50	50	50
10x PCR	50	50	50	50
Taq	~~2~~	~~2~~	~~2~~	2
H_2O	~~375~~	375	371	375
DNA (ng/ul)	1	1	1	1
	500	500	500	500

100 ul / Rxn.

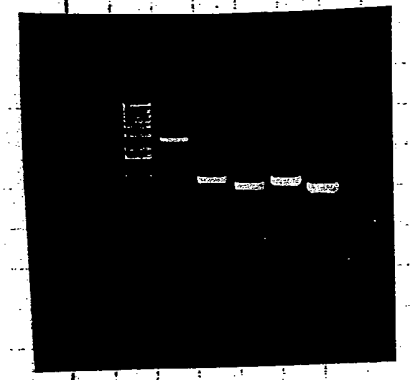
6 HTPA1008 / HTPB411 PD10 / PA2

1/27/95

Run PCR:

95°C	5 min	
95°C	30 sec	] 25X
55°C	30 sec	
72°C	1 min	
72°C	7 1/2 min	
4°C	Hold	

Run 5 ul of Rxn on gel with 1 kb ladder



1	HTPB411	5' Bam/3' Xba	PA2
2	HTPA1008	516p	PA2
3	↓	185bp	PA2
4		516p	PA2
5		185 bp	PA2

Precipitate Reactions
Add equal Vol
13% PEG / NaCl
Spin 10 min
Remove Supernatant
1000 ul 70% Ethanol
Wash pellet
Drop Rinse Supernatant
Dry pellet 5 min. at RT.
Resuspend pellet in 100 ul TE.
Set up Digests

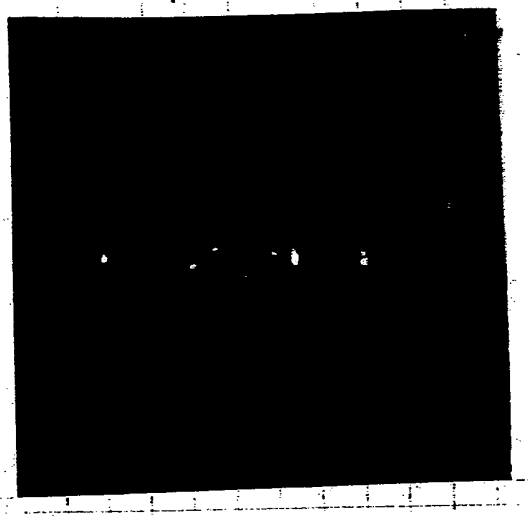
DNA (PCR Rxns)	10 ul
10 X #2 Buffer	5
H2O	34
Bam	0.5
Xba	0.5
	50 ul

Incubate 37°C 4 hrs.
Run on 0.8% LMP gel with
1 kb ladder.

HTPAN08 / HTPB411 PD10 / PA2

1/27/95

Cut out bands.
Take picture.



1	HTPAN08	51 bp	PA2
2	↓	185 bp	PA2
3	↓	51 bp	PD10
4	↓	185 bp	PD10
5	HTPB411	PA2	

Gene Clean fragments
- Resuspend in 40 ul TE.
Set up ligations.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
9111 + 3146	6	6							6								
9112 + 3146			6	6						6							
9113 + 3146					6						6						
9114 + 3146						6						6					
HTPB411 (PA2)							6	6					6				
10 X Buffer	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
H_2O	9	9	9	9	9	9	9	9	11	11	11	11	11	15	15	15	17
T4 Lig (1U/ul)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PD10 B/x 9/10					2	2								2			
PA2 B/x 9/6	2		2				2								2		
PA 2 B/x 1/27		2		2				2								2	

		18x
10X Buffer	2	36
T4 ligase	1	18
H_2O	9	162
	12ul	12ul / Tube

Add Appropriate Ul of Vector / Fragment or H_2O.

1/27/95

Incubate ligations 16°C over weekend

[signature] 1/27/95

1/30/95

Transform ligations

100ul of Chem. Competent Cells
10ul of ligation Rxn.

PA2 Constructs DH5α ⊕ control PA2
PD10 Constructs M15 rep4 ⊕ control PD10

Incubate on ice 1 hr
Heat 42°C 45sec
Place on ice
Add 400ul LB
Incubate 37°C O/S 1 hr.
Plate 200ul & 300ul onto
LB+Amp plates for ligations
1-8 onto 150mm plates
for ligations 9-17 plate 100ul
onto LB+Amp 100mm Plates.

Incubate 37°C O/N.

1/31/95

Plates look good
No colonies on ⊖ Control plates
Colonies on ⊕ Control plates

- Inoculate plates colonies onto LB+Amp
  for PA2 Constructs -
  200 ul of LB+Amp in 96 well dish
- Inoculate colonies onto LB+Amp Kan
  for PD10 constructs
  200ul of LB + Amp in 96 Well Dish

HTPA1008/HTPB411 PA2/PD10

1/31/95

B+Amp:

(1)	9111 #3146 + PA2 9/6	48	Plate #1
(2)	9111 #3146 + PA2 1/27	13	
(3)	9112 #3146 + PA2 9/6	35	
(3)	9112/3146 + PA2 9/6	12	plate #2
(4)	9112/3146 + PA2 1/27	13	
	PA2 9/6	2	
	PA2 1/27	1	
(7)	HTPB411 + PA2 9/6	48	
(8)	HTPB411 + PA2 1/27	12	

LB+Amp/Kan:

(5)	9113/3146 + PD10	48	plate #3
(6)	9114/3146 + PD10	48	

Incubate plate 4 hrs 37°C. with aeration

Setup PCR's.

		70x
9111	1	70
3146	0.1	7
10xPCR	3.2	240
10xdNTP	3.2	240
H_2O	22.35	1564.5
Taq	0.15	10.5
Cult.	2	
	32	30 µl/tube

		55x
9112	1	55
3146	0.1	5.5
10xPCR	3.2	176
10xdNTP	3.2	176
H_2O	22.35	1229.25
Taq	0.15	8.25
Hib.	2	
	32	30 µl/tube

		(50x)
9113	1.0	50
3146	0.1	5
10xPCR	3.2	160
10xdNTP	3.2	160
H_2O	22.35	1117.5
Taq	0.15	7.5
Cult	2	
	32	32 µl/tube

		(50x)
9114	1	50
3146	0.1	5
10xPCR	3.2	160
10xdNTP	3.2	160
H_2O	22.35	1117.5
Taq	0.15	7.5
Cult	2	
	32	30 µl/tube

10 HTPA005 / HTPB411 PD10 / [illegible] DA2

1/31/95

		65X
HTPB411 5'Bam	0.1	6.5
3'Xba	0.1	6.5
10X dNTP	3.2	208
10X PCR	3.2	208
H_2O	23.25	1511.25
Taq	0.15	9.75
Cult.	2	
	32ul	30ul / tube

PCR.

95°C	5min	
95°C	20sec	] 30X
55°C	20sec	
72°C	1 min	
72°C	7 1/2 min	
4°C	Hold.	

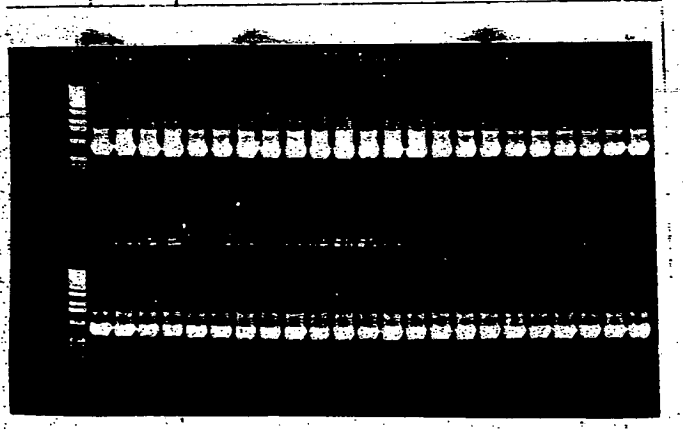
PA2 controls for 9111, 9112 9HTPB411

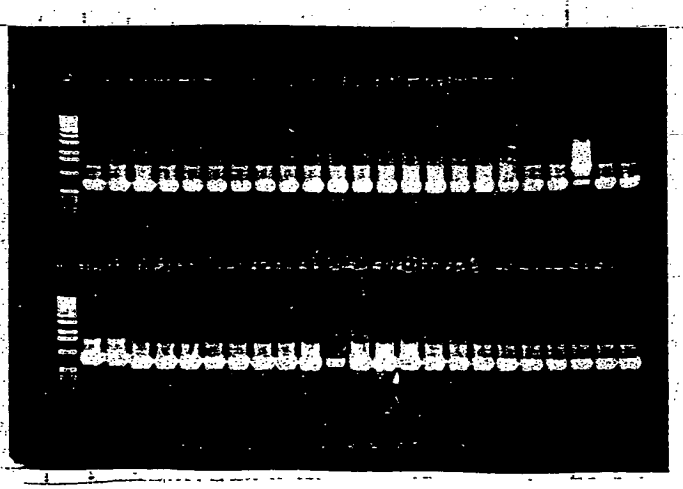
2/1/95

Run Reactions on 1% TAE Agarose gel with 1kb ladder.

9113 A1-D12

9113/9114 E1-H12



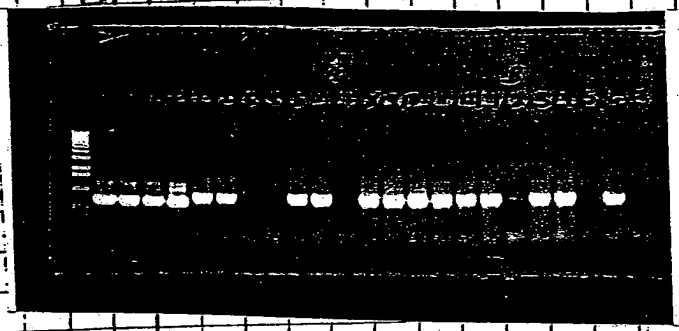


HTPA1008 / HTPB911 + PD10/PA12

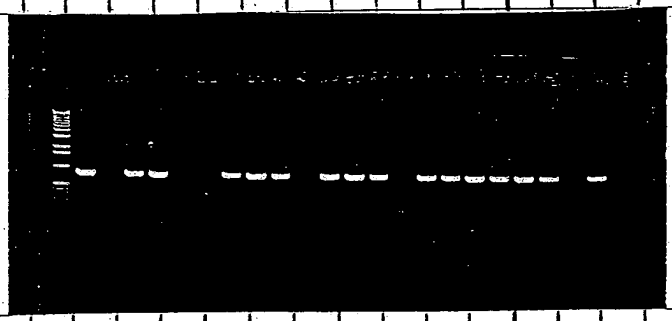
2/195

9111 E1-H12

9111 A1-F1

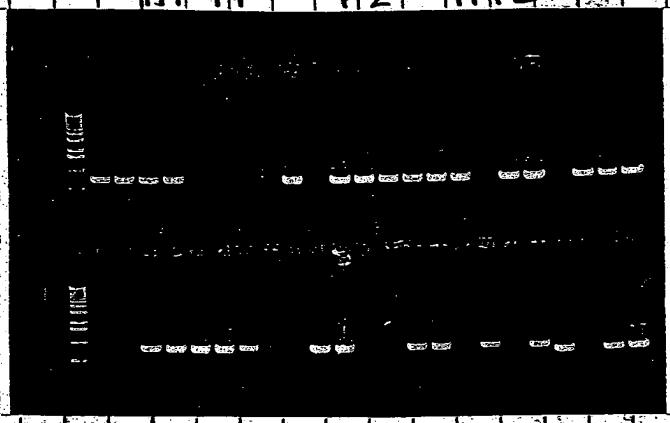


9111 A1-F1

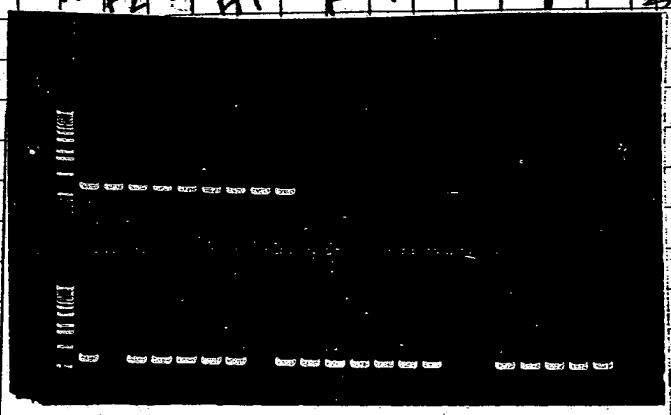


9111 A1-F1

9112 F2-H12

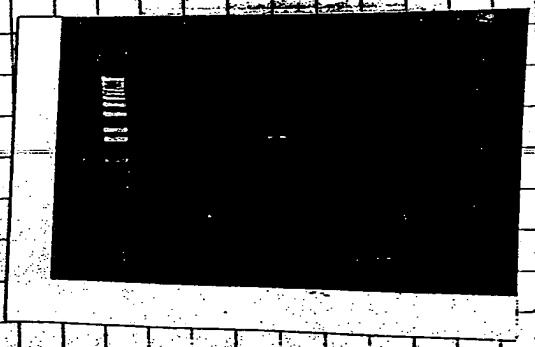


9112 F2-H12 A1-C1 — PA12 controls HTPB911 D1-H12



HTPB911 D1-H12

HTPB911 D1-H12



12 | HTPA1008 | HTPB411 PA2 | ADP

1/1/95

Inoculate 200 µl LB + Amp/Kan
with 20 µl of ⊕ clones to do
small scale inductions (micro)
Incubate at 37°C w/aeration
2 hrs.
Add 5 µl of 100mM IPTG to 2mM IPTG
final Conc.
Incubate 4 hrs 37°C w/aeration
Spin 10 min.
Resuspend pellet in 5 µl H₂O
Add 15 µl 2X Buffer
Store -20°C till tomorrow.

Inoculate 5 ml TB + Amp
with PA2 ⊕ Clones.

① - 11 9N11 / 3146 + PA2 9/6
② - 8 9N11 / 3146 + PA2 1/27
③ - 10 9N2 / 3146 + PA2 9/6
④ - 10 9N2 / 3146 + PA2 1/27
⑦ - 1 HTPB411 + PA2 9/6

Incubate 37°C w/aeration O/N.

2/2/95

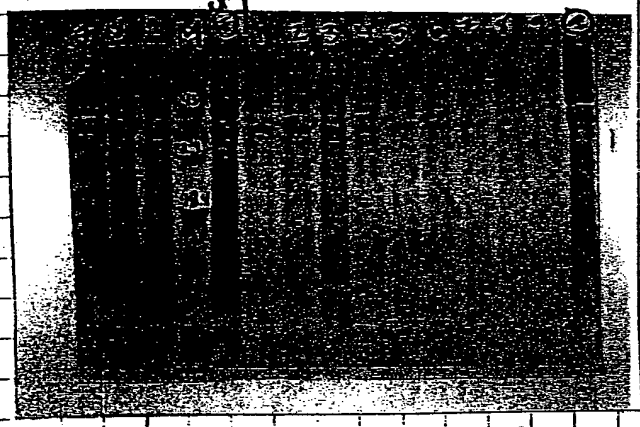
Run Protein gels 15% Stacking
15 µl of Samples + LMW marker.

150V 1½ hrs
Stain 30 min 37°C
DeStain 1 hr 37°C

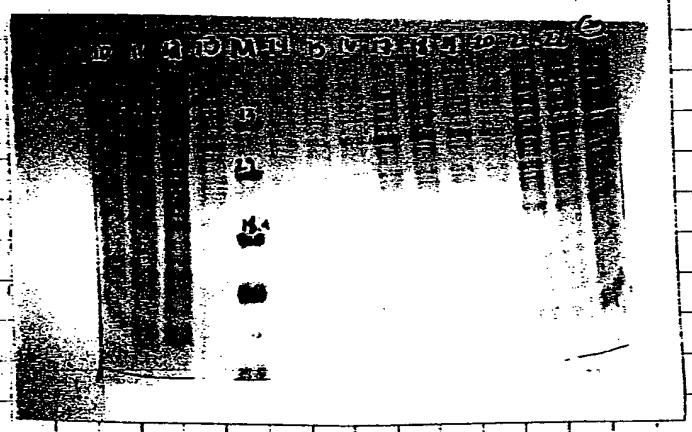
HTPAN08/HTPBY11 PA2/PD10

2/2/95

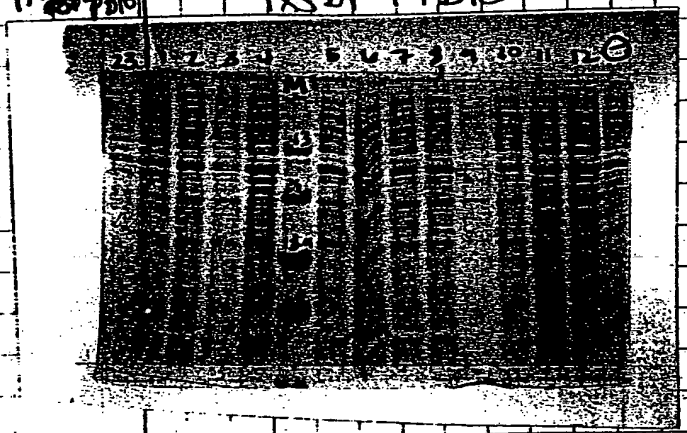
HTPAN0865bp+PD10



HTPAN08 5bp + PD10



HTPAN08 185bp + PD10



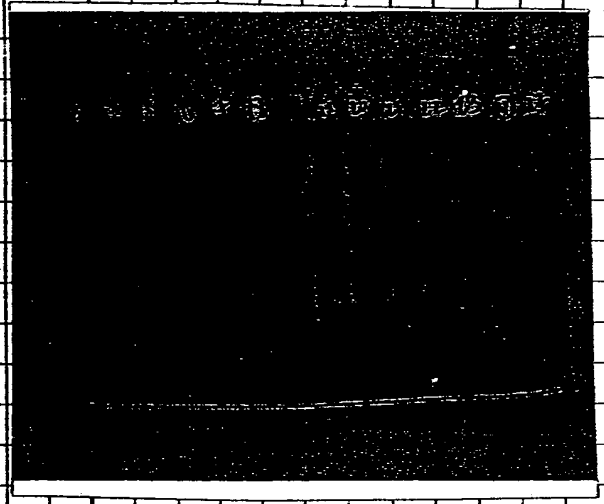
HTPAN0885bp
Should produce a protein 32.5KD

HTPAN08185bp
Should Produce a protein = 27.7K

Looks like HTPAN08 185bp + PD10 — about 90% induction

14 HTPAN08 | HTPB411 PDD / PA2

2/2/95



Looks like all induced except ⊖ control.

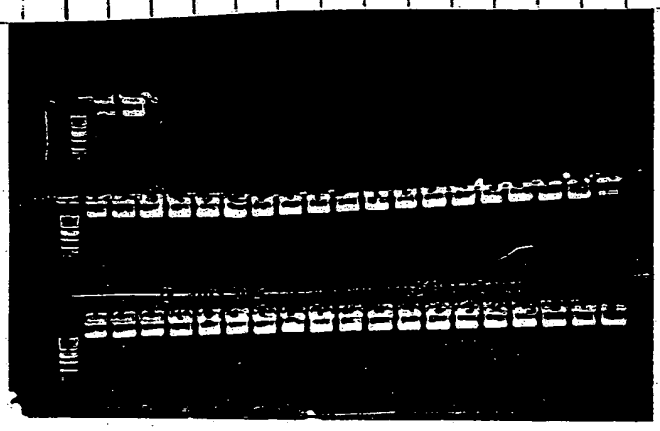
Do large scale preps.

HTPAN02SAL 18SD+PDD

Obj)
Do Boiling Mini Preps. of PA2 Constructs

2ml culture Spin 2 min
Remove Supernatant
Resuspend pellet in 300µl STET + RNase + Lysozyme
Heat 100°C 2 min
Spin 10 min
Remove Pellet
Add 750µl 13% PEG-8000 / 1.6M NaCl
Mix Well
Spin 10 min
Remove Supernatant
Add 1ml 70% Ethanol to Wash pellet
Spin 5 min
Remove Supernatant.
Allow Pellet to dry at R.T. 10 min
Resuspend pellets in 150µl TE
Run 2µl on 1% TAE gel with 1Kb Lab Ladder

2/2/95



Mini preps all look good.

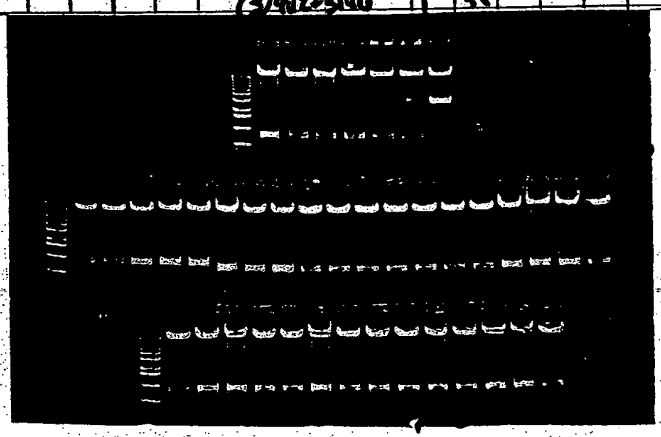
Check for inserts. Big digestion w/ Bam / Xba.

DNA	5 ul
10x	3 ul
H2O	21.8 ul
Bam	0.1 ul
Xba	0.1 ul
	30 ul

Incubate 37°C overnight

2/3/95

Run Dpnl on gel with 1Kb ladder.



All look correct size.
Select 2 to Sequence with internal primers.

(1)	1, 2	RPD6 FP16
(2)	1, 2	
(3)	1, 2	
(4)	1, 2	

HTPB411+PA2 - FP23.

Corrie Checked Seq. Some looked good so did Mini

HTPAN08 / HTPB411 + PA2 / PD10

1/13/95

- Inoculate 3ml TB+Amp w/ Cultures 1-1, 1-2, 2-1, 2-2, 3-1, 3-2, 4-1, 4-2 of HTPB411+PA2. Make Glycerols.
- Inoculate 200ml LB+Amp w/ HTPB411+PA2 to do Maxi Prep
- Inoculate 10ml LB+Amp Kan w/ induced culture of HTPAN08 185bp+PD10 (#15). - Do Large Scale induction

1/14/95

- Made Glycerole Stocks -80°C Protein Expression Box #1

- HTPAN08 185bp + PD10 #12 -
  Inoculate 300ml LB+Amp/Kan w/ 3ml of O/N culture
  Incubate 37°C 3hrs - until OD_{600} = 0.4-0.6
  Add 100mM IPTG to 2mM - 6ml
  Incubate 37°C 4½hrs w/ aeration
  Spin 5K 15 min
  Resuspend Pellet in 30ml of 6M Gn HCl pH8
  Store at 4°C O/N.
- HTPB411+PA2 Maxi Prep

  Reagen Maxi
  Spun culture 6K 20min
  Pour off supernatant

(pg 27)

~~HTBAN08~~ / HTBV11

pg 16

2/15/95

HTBV11 PAC

Resuspend pellet in 10ml of P1 + RNase
Let sit RT 5min
Add 10ml P2 while mixing gently
Add 10ml P3 while mixing
Let sit on ice 20 min
Spin 20 min 8K.
Equilibrate Tip-500 with 10ml QBT
Apply Supernatant to Equilibrated Columns
Wash Column 2x with 30ml QC
Elute DNA in 15ml QF
Add 0.7x (10.5ml) of Isopropanol
Mix Well
Spin 9K for 25min
Pour off Supernatant
Wash pellet with ice cold 70% Ethanol (10ml)
Spin 9K 10 min
Pour off 70% Ethanol
Allow Pellet to Dry at RT
Resuspend pellet in 1400µl TE
Run 5µl on gel
Read OD 260/280 at 1:200 Dilution

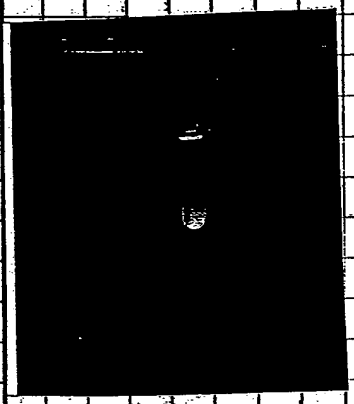
abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm
-0.0063	-0.0028	-0.0022	6.9003	0.1449
0.1519	0.0964	0.0232	1.7591	0.5685

1.52 µg/µl × 1400µl Total

Store 4°C Plasmid Box #2.
Sequence w/ internal primers to confirm sequence

28 HTPB411 & HTPA1508

2/14/95



Both good.
- See if Sequence is good

2/15/95

Digest @ Ling DNA w/ Bam/Xba to see if Insert "Pops" out

DNA (250ng/μl)	4
10x #2	3
H_2O	22.6
Bam	0.2
Xba	0.2
	30

Incubate 37°C.

Submitt for sequencing w/ universal primers: HTPB411PA2 RP/FP

RP01A	RP06A	RP10	FP14	FP18	FP22 A
RP03A	RP07	RP11	FP15	FP19	FP23 C
RP04A	FP08	FP12	FA16	FP20	RP50
RP05	RP09	RP13	FP17	FP21 A	

RP50 Reverse PA2 Primer

HTPAN08 / HTPBY11

Submitt for Sequencing w/ internals 2/13/95
HTPAN08 516p + PA2
HTPBN08 185bp + PA2.

Trans Plasmids to Steve to submit to Protein Expression for Baculovirus.

516bp.			185bp		
RP12	FP14	RP01	FP16	FP17	RP50.
FP13	RP05	FP18	FP14	RP01	
RP10	FP08	RP06	RP05	FP18	
FP16	FP17	RP50.	FP08	RP06	
HTPAN08PA516RP/FP			HTPAN08P18 5RP/FP.		

HTPAN08 185bp + PD10 #12 Large Scale
~~Induction~~ Inductions.
Spin Culture 20 min 8K.
Prepare $NiSO_4$ Column.
Wash Apply 2nd Resin to Column
Wash 20 ml of H_2O.
Add 30 ml 0.1M $NiSO_4$ to Charge
Wash 30 ml H_2O.
Equilabrate with 30 ml Coll Gn HCl pH6
Apply Supernatant - Collect Flow.
Wash 45 ml pH6 - Collect pH6
Wash 45 ml pH6 - Collect pH6
Elute 6 ml pH5 - Collect pH5
Strip 45 ml pH2 - Collect pH2.

Run on 15% Acrylamide Stacking gel.

490 ul H_2O
20 ul Elute Gn HCl prep -
35 ul 50% TCA
50 ul. 0.15M NaDOC.

Mix Well
Spin 10 min

30 HTPB411 & HTPA008

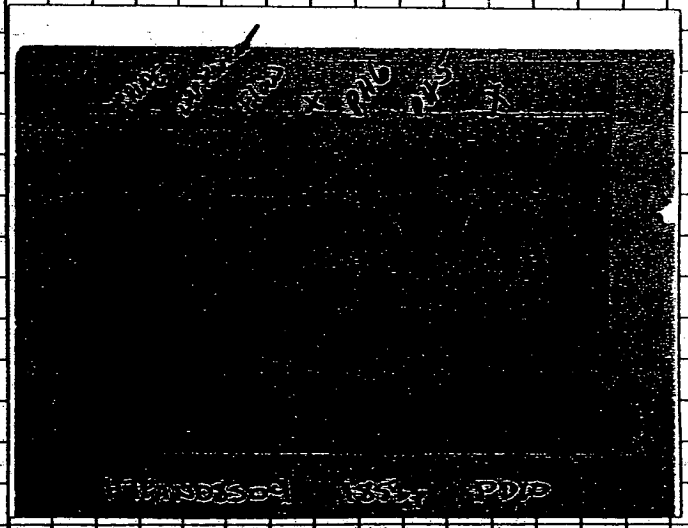
2/15/95

Remove supernatant
Resuspend pellet 10 ul 0.2N NaOH
Add 10 ul 2X Dissociation Buffer
Boil 5 min - lost pH8 + pH12 ← Samples
mixed with water.
Spin 5 min
Run 20 ul on gel with LMW
Protein Marker
@ 150 V 1 hour.

Stain 30 min. 37°C
DESTAIN overnight.

2/16/95

HTPA008 185 PDO #12



Protein looks good

Reapply 3 ml of pH 5.0 to Column
Add 30 ml pH 8
Send to Protein Expression & have renatured over column

Inoculate 500 ml of LB + Amp/Kan with 20 ml of HTPB411 + pQE60

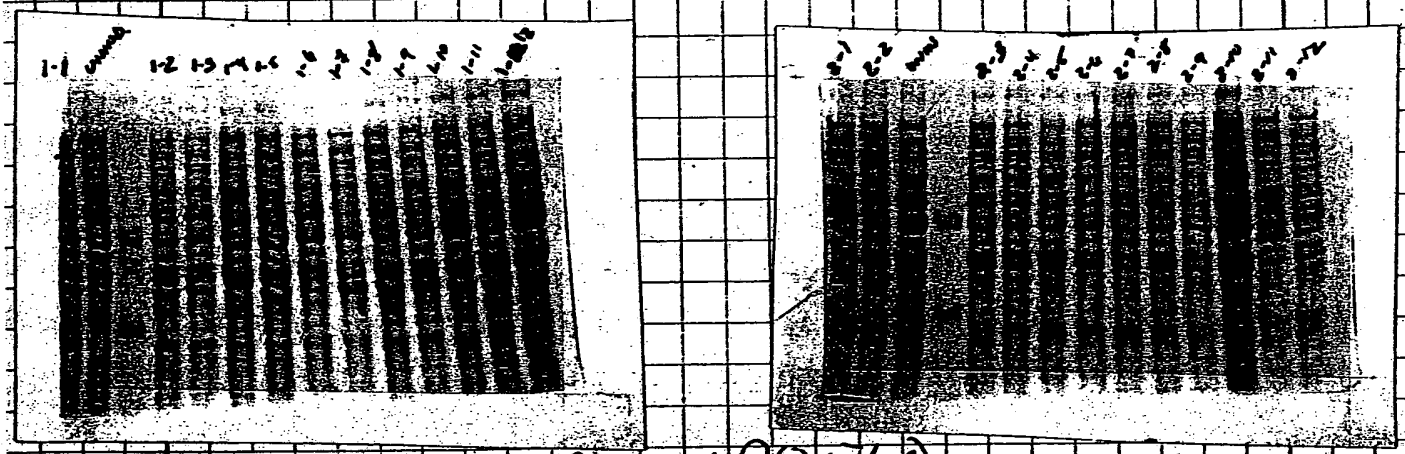
HTB111 & HTBDN08

2/16/95

Incubate 37°C w/aeration
2 hrs.
Add @ 100 mM IPTG to 2mM
10 ul.
Incubate 37°C w/ aeration
overnight.

2/17/95

Spin Cultures 2 min
Remove Supernatant.
Resuspend culture 20 ul H2O.
Add 20 ul 2x Dissociation Buffer.
Heat 100°C 5 min
Spin 5 min.
Run 10 ul on 10% Stacking
gel.
Accidently ran 1 kb Marker instead
of Rainbow Marker.



HTB111 + PQE60

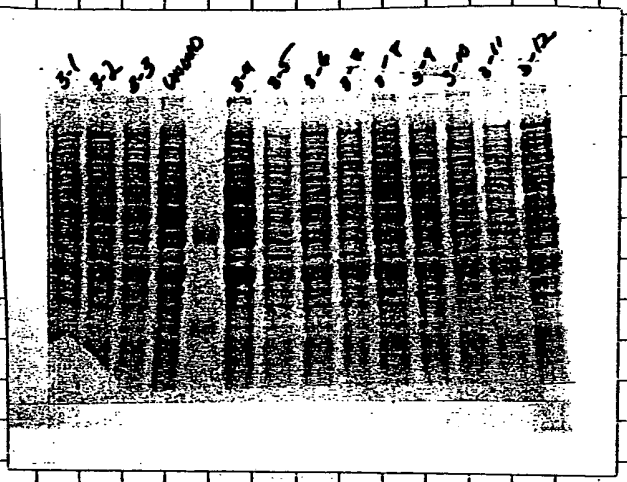
Run 150V 1½hr

Stain
DESTAIN

Ute-A Rule
2/17/95

32 HTPAN08 | HTPBY11

~~12/2~~ 12/4/95



HTPBY11 + POE60

Try growing up 1 for induction - large scale

2/21/95

Inoculate 5ml LBT Amp/Kan
with HTPBY11 + POE60.
2-2 & 3-4
Incubate 37°C.

Transform - HTPAN08 5/bp + PD10 9/13/95
HTPAN08 5/bp + POE60 1/5/95
into M15 Chemically Competent Cells.

Thaw M15 on ice
To 200 µl of Cells add ligations
Incubate on ice 1 hour.

(1943)

H-(WT) + SV

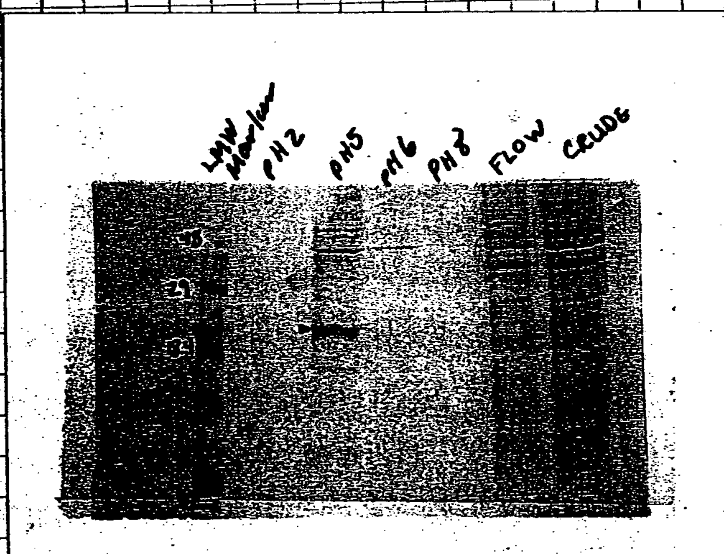
(Pg 26)

2/17/95

Mix well
Spin 10 min
Remove Supernatant
Resuspend in 10 ul 0.2 N NaOH
Add 10 ul 2X dissociation Buffer
Heat 100°C 5 min
Run all on gel.
150 V 1 hour.

STAIN 30 min 37°C
DESTAIN 30 min 37°C

Take Picture.



looks like I have protein.
Looks slightly Contaminated
Try re eluting To try and Clean up prep?
Need Ext in PDEGO

2/21/95

pick 48 more clones from the 2/13/95 into 200 ul of LB Amp/Kan

pg 32

2/21/95

Heat 48°C 45sec.
Place on ice
Add 400 µl of LB
Incubate 37°C 1 hour.
Plate 300 µl onto LB+Amp 150mm plate
100 µl onto LB+Amp/Kan 100mm plate
Incubate 37°C O/N.

2/22/95

PCR HTPAN08 ST + PQE60 48 BE1-H12
HTPAN08 ST + PD10 48 A1-D12
into 200 µl LB+Amp/Kan
Incubate 37°C Shaeration O/N.

HTPB411 + PQE60.
To 300 ml LB+Amp/Kan. Add
3 ml of O/N culture. 2-2 1/3-4
Incubate @ 37° aeration 3 hrs until
OD_{600} ~ 0.4 - 0.6
Add 100 mM IPTG to 2 mM (1.2 ml)
Incubate 37°C 4 1/2 hours
Spin cultures 7K 20 min
Remove supernatant
Resuspend pellet in 30 ml. 6M GuHCl
pH 8.
Store 4°C O/N.

2/23/95

Do PCR of HTPAN08 clones.

HTPAN08 | HTBH11

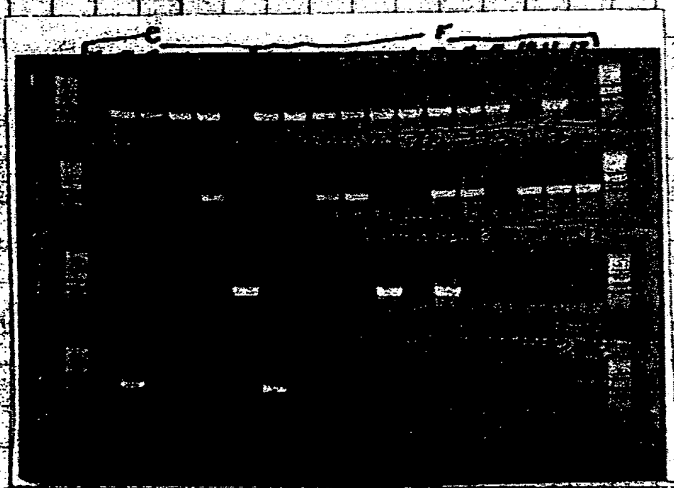
2/23/95

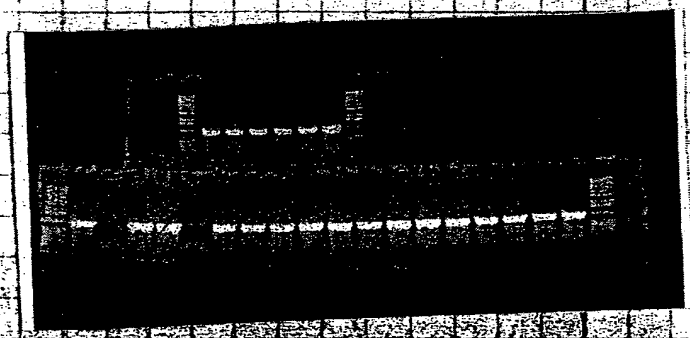
HTPAN08S04 5'bp + PD10		(50X)
9113	1.5	75
B146	0.1	5
10xdNTP	3.2	160
10x PCR	3.2	160
H_2O	21.9	1095
Taq	0.1	5
Culture	2	
		30 µl/tube

HTPAN08S04 5'bp + PQE60		50X
9113	1.5	75
PQE30	0.1	5
10xdNTP	3.2	160
10x PCR	3.2	160
H_2O	21.9	1095
Taq	0.1	5
Culture	2	
		30 µl/tube

PCR Program # 06

Run 10 µl on gel w/ 1 Kb ladder





HTPAN08 PD10 A1-D12
A2, A7, B12, C5, C7, D5, D9, D10
Inoculate 2ml LB+Amp/Kan
with 20 µl O/N culture

HTPAN08 PQE60 E1-H12
E1, E2, F1, F2, G1, G3, H1, H2
Inoculate 2ml LB+Amp/Kan with
20 µl O/N culture
Incubate at RT Overnight.

HTPB#8/ HTPB#11

2/23/95

HTPB#11 + PQE60 2-2 + 3-4
Spin Culture 8K 20 min.
Transfer Supernatant to fresh tube-
(Crude Extracts)

Prepare Columns.
Prepare Columns with 2ml NTA Resin
Wash 30ml H_2O
Charge 30ml 0.1M $NiSO_4$
Wash 30ml H_2O.
Equilibrate 30ml 6M GnHCl pH8.

Apply Supernatant to Columns.
Collect as flow.
Wash 30ml 6M GnHCl pH8
Collect as pH8.
Wash 30ml 6M GnHCl pH6
Collect as pH6.
Elute protein @ 5ml 6M GnHCl pH5
Collect as pH5
Strip Column. 30ml 6M GnHCl pH2
Collect as pH2.

Store 4°C O/N.

2/24/95

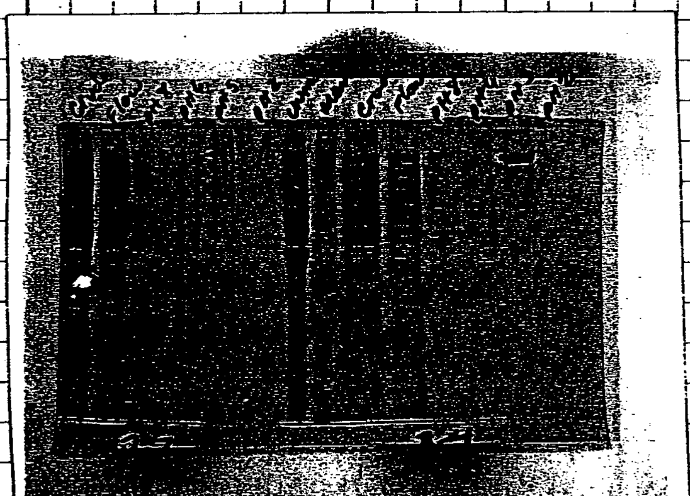
HTPB#11 + PQE60.

80 ul H_2O
400 ul H_2O
20 ul of eluted Protein in 6M GnHCl.
50 ul 0.15% Na DOC.
75 ul 50% TCA
Mix well
Spin 5 min
Remove Supernatant
Resuspend pellet in 0.2N NaOH. ~10 ul

 HTPAN506 | HTPB411

2/24/95

Add 10ul 2X Dissociation Buffer
Heat 100°C 5min
Spin 1 min
Run 15ul on gel with Uninduced
and Rainbow marker
12.5% Acrylamide Stacking gel
150V 1 hour.



STAIN 37°C
30 min
DESTAIN 30 min
37°C

Take Picture

looks like isolated
protein

Store at 4°C
Ask Steve about
how to get
[illegible] renature

HTPAN08 51bp +PAE/PD10

Place tubes at 37°C w/aeration
for 2hrs
Add 100mM IPTG to 2mM 4ul
Incubate 37°C 4hours
Spin 1ml 2min
Remove supernatant
Resuspend pellet in 30ul H_2O.
Add 30ul 2X Dissociation Buffer
Run 15ul on gel with ~~Rainbow~~ LMW Marker
150V 1hour

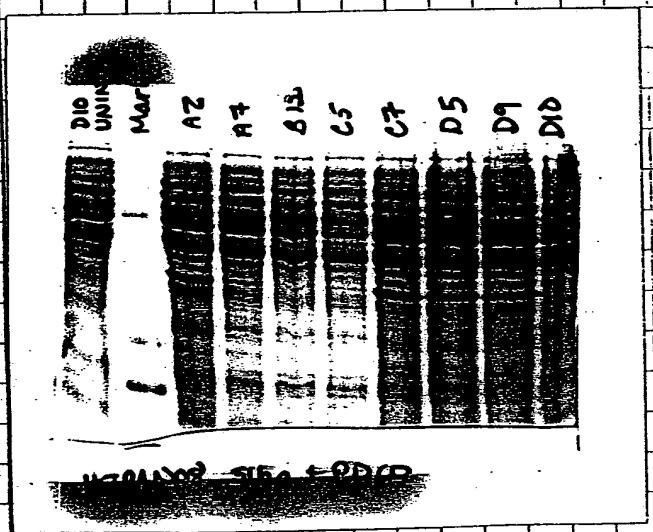
HTPA408 | HTPB411

2/24/95

Stain 30 min 37°C
DESTAIN over weekend at RT

2/27/95

Take picture

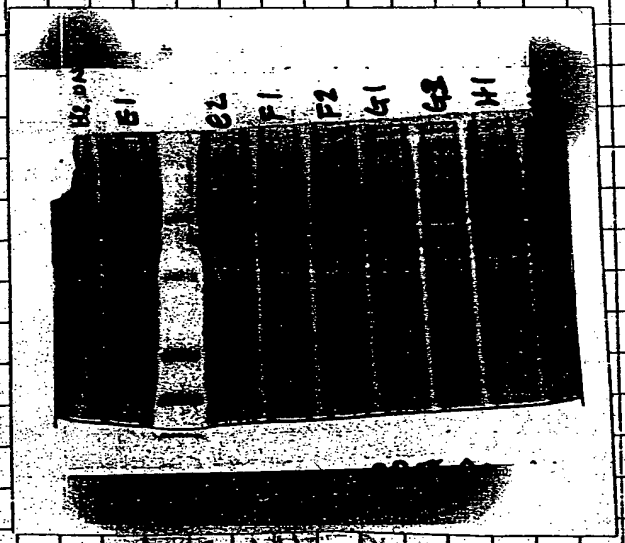


A2
C7
D5
D9

Uninduced

Slow up to make Glycerol. Pick 1 to Do Large Scale Prep
C7 - large Scale
Inoculate 5ml LB + Amp / Kan w/ C7. Incubate o/n w/ aeration. Make Glycerols of A2, C7, D5, D9
Does not look like anything Induced -)

Try Running Again - Hopefully will Work this time



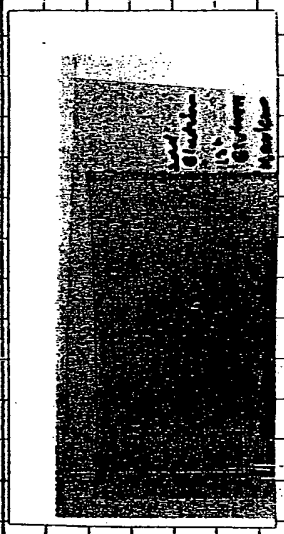
48 HTPAN7/HTPB4

2/27/95

Re Run HTPAN08 51bp + PCR(2)
10 µl.

Run 10µl each of the 1st + 2nd
Imidiate elution of HTPAN08 185+PD10
#12

2/28/95



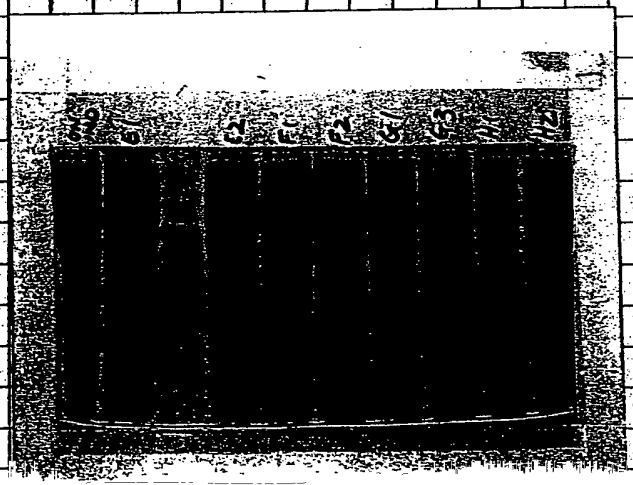
HTPAN08 185bp + PD10 #12.

looks like both fractions
have protein.

~~HTPAN08 185bp + PD~~

Inoculate 300ml LB +
Amp Kan with
HTPAN08 51bp + PD10 #7
to do Qiagen Maxi
Incubate 37°C & O/N.

Re Run HTPAN08 51bp + PCR(2)
150V 1 hour
Stain / DESTAIN



Does not look like
there was any
induction
Try other clones
that lit up

F 4,5,6,8
F 3,4,5,6
F 4,6,7,8
H-B 4,5,6,7

Inoculate 2ml LB+

HTPAN08 / HTPBY11

2/28/95

Water 20 μl of culture from 2/23 (pg 44). Let sit at RT O/N

HTPAN08 51bp+PD10 C7 Qiagen Maxi - See pg 42 - Ded along side O/L 6 SV PQE60.

HTPAN08 51bp+PD10 C7
Large Scale Induction
Inoculate 300 ml LB+Amp Kan
5 ml of O/N culture.
Incubate 37°C w/ aeration
until OD 600 = 0.4 - 0.6 ~2½ to 3 hours
Add 100mM IPTG to 2mM (6ml)
Incubate 37°C w/ aeration
4½ hours.
Spin culture 5K 15 min.
Pour off supernatant.
Resuspend pellet in 30ml
6M Gn HCl pH8
Store 4°C O/N.

3/1/95

Incubate HTPAN08 51bp+ PQE60
in 2 ml LB+Amp Kan 37°C
w/ aeration
Incubate until ~ 2 hours
Add 100mM IPTG to 2mM (4 μl)
Incubate 37°C 5 hours
Spin 1 ml culture
Remove supernatant
Resuspend pellet in 40 μl H_2O
Add 40 μl 2X Dissociation Buffer

[signature]
3/1/95

H11ΔN | H11PBY

3/1/95

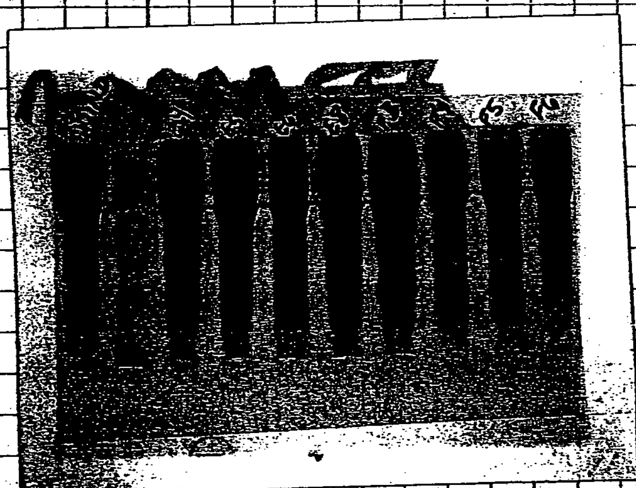
Heat 100°C 5 min
Spin 2 min
Run 10ul on gel w/ LMW/Marker
180V 40 min
12.5% gel

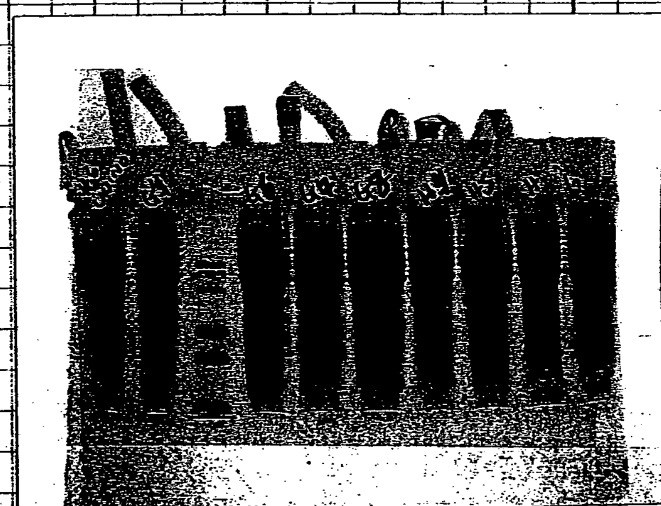
Stain O/N -

~~8/2/95~~
3/2/95

~~DESTAIN~~
to
DESTAIN.

Take Picture





Nothing induced -

60 New Clones - HT4A Screen

3/7/95

Spin through G25 Column.
Count Load.

HT4AI33	41	1	899759.00	0.21	1.00
HT4CM40	42	1	965819.00	0.20	1.00
HT4CB94	43	1	1254146.25	0.20	0.80
HTABG14	44	1	1446598.62	0.20	0.70

Add 100 µl of Salmon Sperm DNA
Heat 100°C 5min
Quick Chill.

Pour off Prehyb
Add 50 ml Hyb Buffer
(2 x PIPES / 10% Dextran SO4, 50% Form)
Add Probe to hyb Buffer
Incubate 42°C O/N

3/8/95

Wash Filters.
Pour off hyb
Rinse filters 0.2xSSC / 0.1% SDS.
Wash filters 65°C
0.2 x SSC / 0.1% SDS.
Wash 3X
Put on film
- 1-20A - HT4AI33
21-40B - HTABG94
The others leave washing at 65°C.
Not enough Cassettes.

3/9/95

Develop film
Place remaining filters on film to expose O/N at -80°C

IL-6SV / HTPAN0851bp + PDE10

pg 42 pg 50

3/1/95

Add 0.7 Volumes Isopropanol 10.5ml.
Mix well
Spin 8K 30min.
Wash pellet 15 ml 80% Ethanol
-20°C.
Spin 8K 10 min
Pour off allow pellet to Dry at RT
20 min.

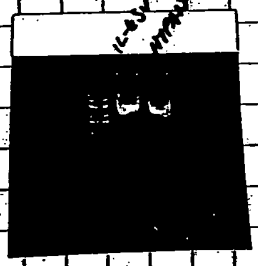
Resuspend pellet in total of 400ul TE and transfer to eppendorf tube.

Read OD260/280 - 1:200 Dilution.

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
1 IL-6SV PDE10	0.1502	0.0995	0.0219	1.6537	0.6047	1.5ug/ml
2 HTPAN0851bp PDE10	0.1091	0.0715	0.0144	1.6582	0.6031	1.1ug/ml

Run 2ul on gel w/ 1Kb ladder

Plasmid looks good
Store Plasmid #2.
4°C



abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm
0.0942	0.0638	0.0201	1.6947

.094 ug/ul

3/8/95

Start Culture to do Dot Blot Induction of HTPAN0851bp + PDE10

Inoculate 30ml LB Amp Kan with C-7
Incubate 37°C ON

62 HTPAN08 51bp PDID -

3/9/95

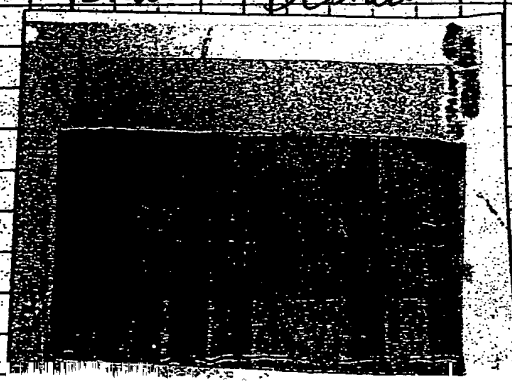
Inoculate 300ml LB+Amp/Kan
10ml of O/N Culture of
HTPAN08 51bp + PDID @ 37°C
Incubate 37°C w/ aeration
until OD_{600} ~ 0.4 - 0.6
Add 100mM IPTG to 2mM (1ml)
Incubate 37°C 4 hours.
Spin 8K 20min
Pour off Supernatant
Resuspend pellet in 30ml 6M Gn HCl
pH 8
Store 4°C O/N.

3/10/95

Spin Culture 8K 20 min
Transfer supernatant to fresh tube.
Run on gel.

400ul H_2O
20ul Protein in Gn HCl
50ul 0.15% NaDOC
5ul 50% TCA.

Mix well
Spin 10 min
Remove Supernatant
Resuspend pellet in 10ul 0.2N NaOH
Run on 2.5% gel.
Stain Destain



looks good -
- Load more
Columns

PD 7/1

pg 60

3/10/94.

Develop film for.
21A-40A HT4CM40
1-20B HT4CB44.

3/13/94

Pick positive clones of
HT4A133
HT4CM40
HT4CB44
into 20ml 5M on 96 well dish
Pick 48 from each clone
Incubate samples at RT O/N.

Plate HTA# for screening of
HTABGF9H.

Dilute 1/100 use 20ul into
6ml LE392 cells OD_{600} = 1.0
Incubate 37°C 15 min
Plate into 150mm NZY plates on
5ml LB + 0.75% Amp. Let set
Incubate 37°C 5 hours.
Store 4°C O/N.

3/15/95

for the 48 clones in 5M of
HT4A133
HT4CM40
HT4CB44
To 50ml LE392 OD_{600} = 1.0 - add 20ul
of phage.
Incubate 37°C 15 min.
Add 15ml NZCYM Broth . . .

HTPAN08 51bp+PD10

pg 62

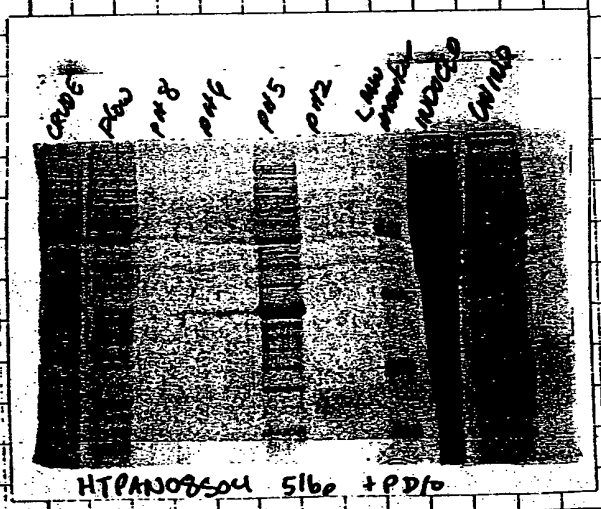
3/13/95

Prepare $NiSO_4$ Column.

2ml Resin		
Wash	30ml	H_2O
Strip	20ml	6M Gn HCl pH 2.
Loden	30ml	H_2O
Charge	30ml	0.1M $NiSO_4$
Wash	30ml	H_2O
Equilibrate	30ml	6M Gn HCl pH 8.
Apply supernatant		- Collect - flow
Wash	30ml	pH 8 6M Gn HCl - Collect
		pH 8
Wash	30ml	6M Gn HCl pH 6 - Collect pH 6
Elute	6ml	6M Gn HCl pH 5 - Collect
Strip	30ml	6M Gn HCl pH 2 - Collect

3/14/95

Run 20ul on gel with the unreduced indicated fractions.



HTPAN08SO4 51bp +PD10

Protein looks good.

HTPAN08 51bp+PD10

3/15/95

prepare 12.5% Preparative gel

15 mm

H TPANO8 868 51bp +PD10

3/15/95

Precipitate 800 ul of pH 5 in H_2O/50% TCA and 0.15% NaDOC
Add 400ul of 0.2N NaOH
Add 400ul 2X Dissociation Buffer
Heat 100°C for 5min
Spin 2min
Run on gel. 100V.

Cut off Marker & Part of gel.

Stain DESTAIN

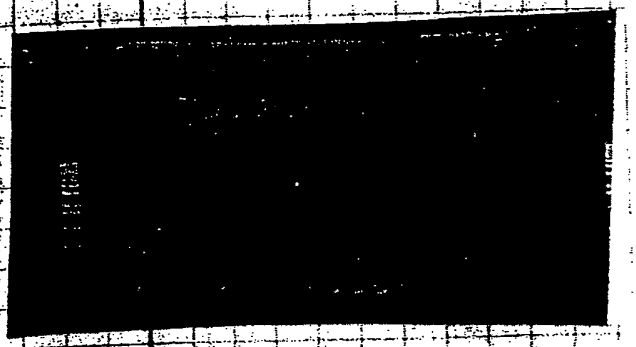
Align w/ corresponding gel.

Cut out from unstained gel.
Place in 15ml conical
Ready for Ab Production

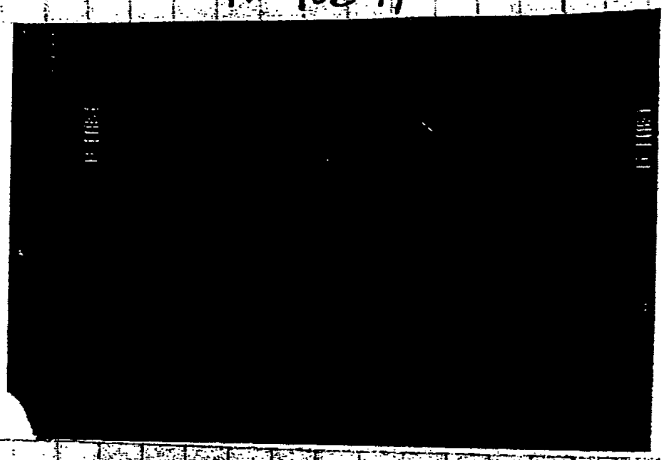
pg 133

HTY / HTA screen

3/16/95

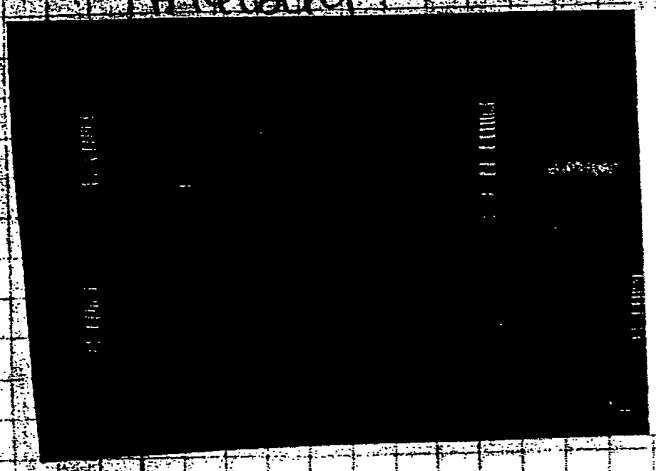


HT4CM40



HT4CM10

HT4CB40



HT4CB44

HT4CB44

Wash HTA & HTY filters
0.2x SSC / 0.1% SDS. - 3x 65°C
Put on film
-80°C O/N.

3/17/95

Develop film.

Random Prime Probe - HSSEN37, HT4SB02, HSKBN09

4/6/95

Mix by flicking
Quick Spin
Incubate 37°C 10 min.

For HSSEN37 use Primer 1

Primers	10ul
DNA	4ul
H_2O	20ul
	34ul.

Heat 100°C 5min.
Quick Chill
Quick Spin
Add 10ul 5x dCTP Buffer
5ul 32P dCTP
1ul Klenow

Incubate 37°C 10min
Put through G 25 Column.
Count 2 doves.
* Did Not put HT4SB02 HSSEN37 HSKBN09 Through Column.

CPM	2SIG%	
2391106.50	0.19	HSKBN09
3005574.25	0.19	HT4SB02
1012483.00	0.20	HSSEN37

Add 100ul Salmon Sperm DNA
Heat 100°C 5min
Quick Chill

Dan M Parker
4/6/95

Screen HOAAH/HSSEN/HSKBN/HT4A4/HT4CB

4/27/95

Inoculate 30 ml TB+Amp
with HTAB494, S01, S02, S03, S04, S05 & S06
Incubate 37°C O/N.

From plated lesions - pick 6 white colonies into 200 ml LB+Amp
Incubate 37°C 4 hours.
Do PCR

HOAAH62	
FP50	1
M13R	0.05
10x	3.2
10x	3.2
H_2O	22.4
Taq	0.15
Cult	2
	32

HSSEN37	
FP50	1
M13R	0.05
	3.2
	3.2
	22.4
	0.15
	2
	32

HSKBN09	
FP50	2
M13R	0.05
	3.2
	3.2
	21.4
	0.15
	2
	32

HT4A480	
FP04	1.2
M13R	0.05
10x	3.2
10x	3.2
H_2O	22.2
Taq	0.15
Cult.	2
	32

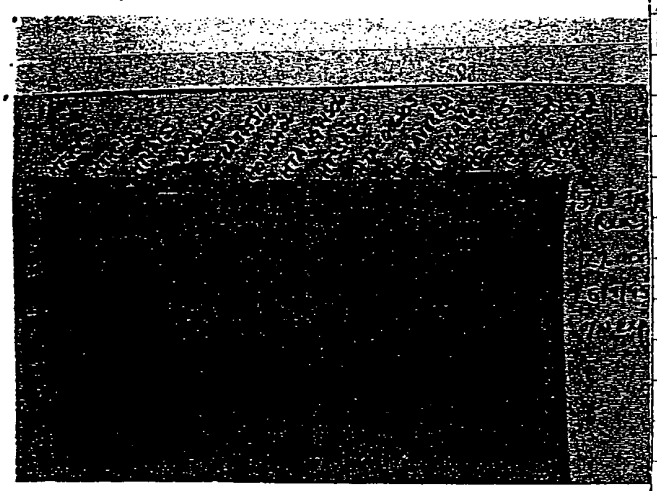
HT4CB44	
FP02	1
	0.05
	3.2
	3.2
	22.4
	0.15
	2
	32

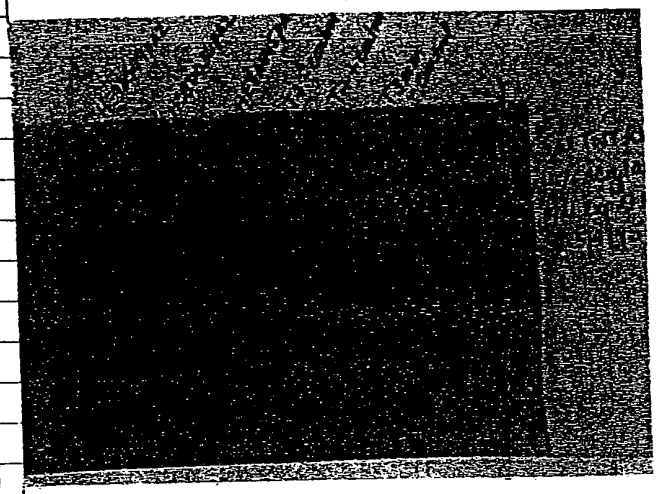
PCR Proj #6

	95°C	5 min
30x	95°C	20 sec
	55°C	20 sec
	72°C	1 min
	72°C	7 min
	4°C	Hold

Ann Reed
4/27/95

5/5/95





5/8/95

1972

Inoculate LB+Amp Kan with HTPAN08 5/6p A7G in PDJO into 100ml.
Incubate 37°C O/N.

Inoculate 250 ml LB+Amp with PREPARE H.TABG 94506 for Maxi Prep
Inoculate 30ml LB+Amp with HOAA11G4 50@2

Incubate 37°C O/N W/Duration

5/9/95

Inoculate 1 lot LB+Amp Kan with 50ml O/N culture of HTPAN08 5/6p A7G in PDJO
Incubate 37°C until OD_{600} = 0.4-0.6
Add 100 mM IPTG to 2mM → 20ml

134 Maxi HTABG94SOC. | Midi HOAAHE2 SO2

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Incubate 37°C 4½ hours
Spin 5K 20 min
Pour off Supernatant
Resuspend pellet in a total of 100 ml
10 mM GuHCl pH 7.8
Let sit O/N at 4°C.

Assay Maxi Prep
by HTABG94SOC
@ 1:200 Dilution

abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm
0.1548	0.1071	0.0400	1.7092	0.5851

1.55 ug/ml

Run 0.5 ul @ on gel.

HOAAHE2 SO2 Alkylin Lysis #1

Spin Culture
Pour off Supernatant
Resuspend pellet in P1 @ + RNase
(from Qiagen) - 2 ml
Let sit RT 5 min
Add 2 ml P2
Mix gently
Let sit RT 10 min
Add 2 ml P3
Mix Well
Let sit on ice 10 min
Spin 20 min 8K
Transfer Supernatant to fresh tube
Add Isopropanol 0.7 Volumes = 4.2 ml
Mix well
Spin 30 min 8K

HTPAN08S04 5'bp ATG + PD10

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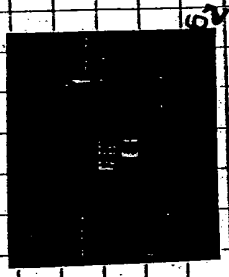
Pour off Supernatant
Wash pellet 70% Ethanol
Spin 10 min 8K
Allow pellet to Dry at RT O/N

5/10/95

HOSAH62S02
Resuspend pellet in 1ml TE
Transfer to 2 Micro fuge tubes
Add equal Volume 13% PEG/1.6M Nacl
Mix well
Spin 15 min
1X 70% Ethanol Wash
Resuspend pellet in a total of 20ul of TE
Run 1ul on gel -
Read OD260/280 1:200 Dilution

OD_{240}	OD_{280}	OD_{320}	$\frac{260}{280}$	$\frac{280}{260}$
0.0279	0.0168	0.0034	1.8322	0.5458

0.2ug/ul.



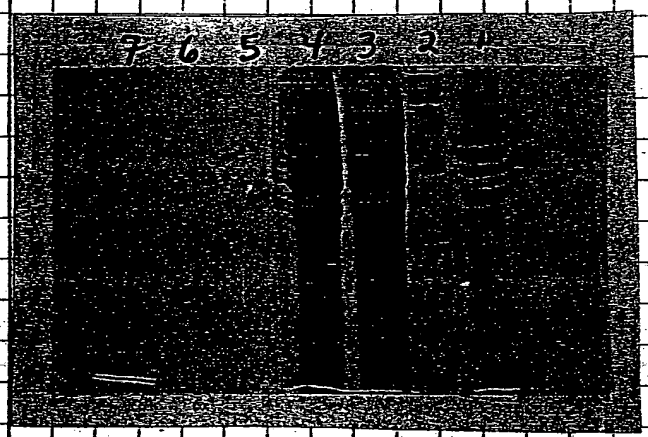
HTPAN08 5'bp ATG + PD10.
Spin 8K 20 min
Transfer Supernatant to fresh tube.
Prepare NiSO4 Column.
Pour 3ml Resin into Column.
Wash 20ml H_2O.
Equilibrate 20ml 6M Gn HCl pH8
Pour on Supernatant. (Crude extract)
and Collect Flow through.
Wash Column 60ml 6M Gn HCl pH8
Collect - pH8.
Wash Column - 60ml 6M Gn HCl pH6.
Collect pH6
Elute Protein - 5ml 6M Gn HCl pH5
Collect pH5

136 HTPAN08504 61bpA TG + PDD

5/10/95
1 Strip Column 30ml 6M Gn HCl pH 2.
collect pH2.

5/11/95

Run Protein Samples on 12.5% Gel
To samples in 6M Gn HCl
450 ul H_2O
50 ul of Sample
50 ul of 0.15% Na DOC
25 ul 50% TCA
mix well
Spin 10 min
Remove Supernatant
Resuspend in 10ul @ 0.2N NaOH
Add 10ul 2X Dissociation Buffer
Heat @ 5 min 100°C.
Spin
Run Samples on 8% SDS-PAGE Stacking gel. 150V 1½ Hours



1 - Uninduced
3 - Crude Extract
4 - flow through
5 - pH8
6 - pH6
7 - pH5

2 - Rainbow Marker

Does not look good try reinoculating culture and do another induction

Fragment preps. + Northern Blots

5/17/95

Set up Digests of Unknowns to give to Brent Kreider - He'll do Northerns.

Clone ID	Conc	5ug DNA	H_2O	10X #2	XhoI	EcoRI
HSKBN09.	2.06 ug/ul	2.5	42.1	5 ul	0.2	0.2
HNBAA26	0.64 ug/ul	7.5	37.1		0.2	0.2
HILB4B0	0.5 ug/ul	10	34.6			
HTHAI33	PCR Product.	20	24.6			
HTHCM40	0.4 ug/ul	11	33.6			
HTHCB44	1.2 ug/ul	4.2	40.4			
HNFAA64	0.73 ug/ul	6.8	37.8			
HTHA480	0.34 ug/ul	15.6	29			
HTABG94	0.99 ug/ul	5.2	38.4			

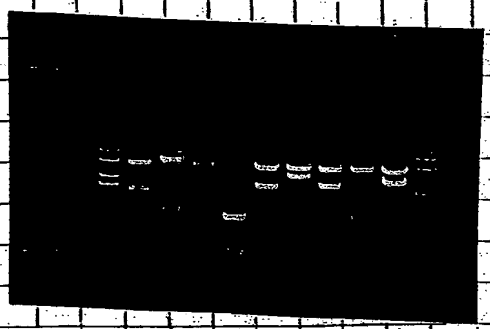
Digest 37°C O/N.

5/12/95

Run 5ul on gel with 1Kb marker.

XhoI/EcoRI Digests.

1 - HSKBN09.
2 - HNBAA26
3 HILB4130
4 HTHAI33
5 HTHCM40
6 HTHCB44
7 HNFAA64
8 HTHA4A480
9 - HTABG94

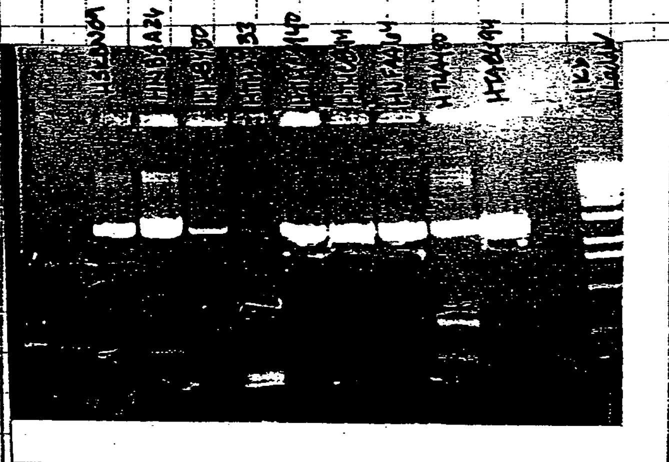


5/12/95

Fragment preps - Northern Blots

5/12/95

Run on 0.8% LMP Gel with 1Kb ladder.
Run 80V 2½ hours.



Cutout fragments
- place into 1.5ml microfuge tube
Store 4°C over weekend.

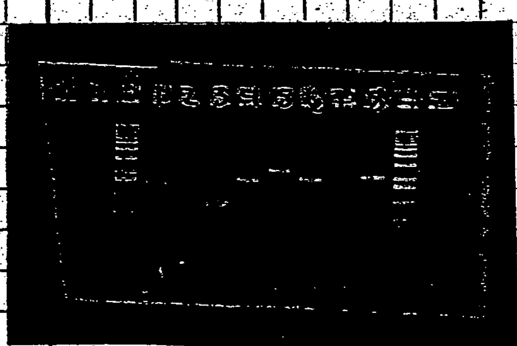
Need to digest HILB430 Again.

HTPAN08 51bp ATG in PD10
- INDUCTION
- Resuspend in 40 ml 6M Gn HCl pH8.
- Store 4°C over weekend.

5/15/95

Gene Clean fragments
Resuspend in 20 µl TE.

Run 1µl on gel with 1Kb ladder



1 HSKBN09
2 HNBAA26
3 HT4AZ33
4 HT4CM40
5 HT4CB44
6 HNFAA64
7 HT4AY80
8 HTABG94
9

Fragments Preps - Northern Blots

5/15/95

Send to Brent Kreider - Kind Kovacs

Clone ID	Libraries Expressed	Size in Kb Eco RI/Xho I		Approx. Conc(ng/ul)	
HSKBN09	HT4, HSK	~1.4	AMK	~100	15ul
HNBAA26	HBJ, HBM, HCA, HNB, HRG, HTO	~0.800		~100	↓
HILBY30	HIL, HLM	~0.60		Will Send 5/17	
HT4AI33	HT4	~0.90		~150	15ul
HT4CM40	HT4	~1.7		~150	
HT4CB44	HT4	~2.5		~150	
HNFAA64	HNF, HSI, HSK, HTA	~1.80		~150	
HT4AY80	HT4, HTX, HT3	~ 0.85		~50	
HTABG94	HTA	~1.7		~150	
HT4CI56	HT4	~1.7		~100	
HT4AI55	HT4	~1.7		~140	
HT4CL32	HT4, HT5, HT3	~1.1		~150	
HT4CA46	HT4, HT3, HCE, HGO, HTA, HL3, HMW	~1.7		~250	↓
HMSAF22	HMS, HOS, HHF, HSR HTN	~2.0		~100	0.5ul
HT3SB70	HNF, HT3, HT4, HT5, HTA	~1.5	CLF	~300	5ul
HT4SB02	HET, HGL, HHF, HSU, HT4, HTE, HTP	~1.3	AMK	~200	10ul

Set up Digest of HILB430 PCR product

DNA	20ul
10x B2	5
H_2O	24.6
EcoRI	0.2
Xho I	0.2
	50ul

Digest at 37°C Overnight

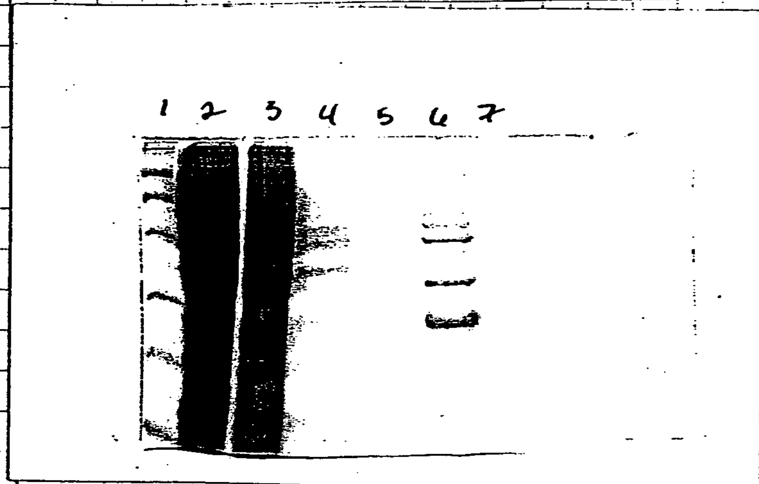
HTPAN08 51bpATG + PD10
Put over Column
Collect Flow through
pH 8
pH 6
pH 5

HTPANO8 SO4 516bp ATG + PDI8

(pg 136)

5/17/95 Run HTPANO8 Samples on 12% Acrylamide gel

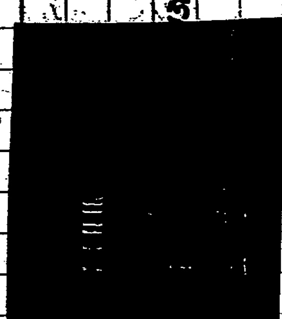
1 Rainbow Marker
2 Crude Extract
3 flow through
4 pH8
5 pH6
6 pH5
7 pH2.



Store at 4°C.

Carrie Did Gene clean of HTLBY30 Xho/RI

Run 1ul on gel with 1Kb ladder



Give to Brent
~600bp
~100ng/ul.

HTPAN0858Y 5'bp ATG tPD10

5/18/95

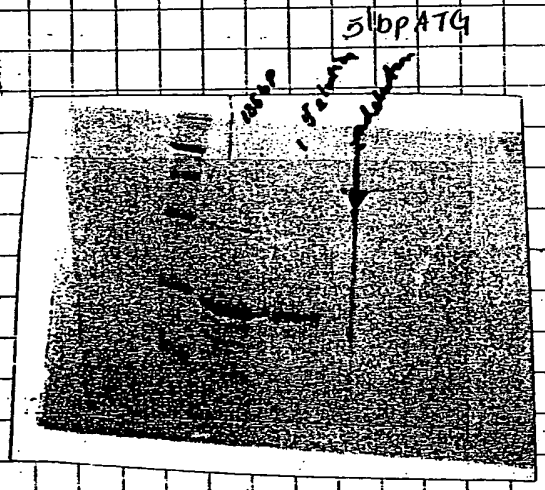
Reapply pH5 of HTPAN0858'bpATG to fresh Column. (from 3/31/95 - pg 71)
Add 3ml pH5 + 20ml pH8.
Wash 30ml pH8.
Wash 30ml pH6.
Give to protein expression for renaturation over column.

5/19 - 5/22

Computer

5/23/95

Removed Column Back
Elute 2ml Imm. Imidazole elution Buffer.
2 times - Run on 12% Gel
with Marker + HTPAN0858'bpATG



5/23, 5/24
5/25

Computer

pg 28 Book #116 Mix #10

5/26/95

Off.

5/18/95

Reapply pH5 of HTPAN08 51bpATG to fresh Column. (from 3/31/95 - pg 71)
Add 3ml pH5 + 20ml pH8.
Wash 30ml pH8.
Wash 20ml pH6.
Give to protein expression for renaturation over column.

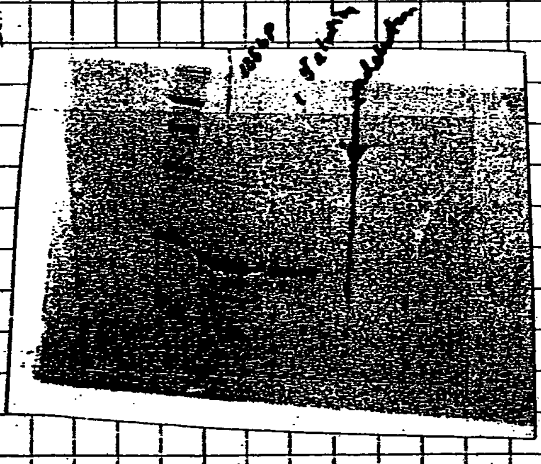
5/19 - 5/22

Competition

5/23/95

Received column back
Elute 2ml Imidazole elution Buffer.
2 times - Run on 12% Gel
with Marker + HTPAN08B 51bpATG

51bpATG



5/23, 5/24
5/25

Competition

5/26/95

Off.

Pg 28
Book #715
AMX #10